CLIN. CHEM. 40/7, 1376–1384 (1994)

Technical and Scientific Developments in Exposure Marker Methodology

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Recent advances in techniques to measure markers of exposure to environmental toxicants in humans are changing the ways in which environmental scientists, epidemiologists, and policymakers characterize and interpret such exposure. In this article we review some major technical and scientific developments in exposure marker methodology for estimating internal dose, with special reference to studies conducted at the US Centers for Disease Control and Prevention. We consider important characteristics of laboratory methods, advances in laboratory technology, analytical standards, and quality assurance of laboratory measurements; comparisons with indirect methods for estimating exposures, such as exposure indices and questionnaires; human pharmacokinetic data; sampling problems; surveillance of human exposures to toxicants; and interpretation of measurements. With a view to increasing the reliability of exposure assessment, we make recommendations for obtaining more data on human exposure to toxicants.

Indexing Terms: *surveillance of toxicant exposures/environmental toxicants/standards for environmental toxicants/dioxin exposure/cotinine, urinary/cotinine, serum/blood lead*

Research in environmental health is focused on finding ways to reduce morbidity and mortality resulting from exposure to hazardous substances. For such disease to be prevented, human exposure to hazardous substances must be minimized. This is a formidable task, because during the course of daily life people come into contact—through breathing, drinking, eating, and touching—with thousands of man-made chemicals, singly or in combination, for which there are few toxicological data from humans or experimental animals. Preliminary cross-sectional studies show that most persons living in industrialized countries have detectable body burdens of many environmental toxicants (e.g., lead, polychlorinated biphenyls, pesticides). Determining and evaluating the exposures received by individuals or populations is crucial to all risk assessments, epidemiologic studies, industrial hygiene and prevention, and public health emergency response systems in the area of environmental health.

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Nonstandard abbreviations: TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; VOCs, volatile organic compounds; NHANES, National Health and Nutrition Examination Survey; NHEXAS, National Human Exposure Assessment Survey.

Received February 7, 1994; accepted April 29, 1994.

In Fig. 1 we depict the general sequence of events leading from the release of toxicants into the environment to the development of health effects in humans (*1*). Initially, a toxicant is released into the environment from a source, such as an incinerator, and then it may be transported to a person's or population's immediate environment (i.e., microenvironment) via soil, air, water, or food. When people come into contact with the contaminated environmental media, an "exposure" occurs, and the toxicant may or may not enter their bodies. One operational definition of exposure is "an event consisting of contact at a boundary between a human and the environment at a specific toxicant concentration for a specified interval of time" (*2*). Although "dose" is the term used for quantifying exposure to a toxicant, the term is used in three different contexts: (i) the *external dose* is the concentration of a toxicant in the environmental media in direct contact with humans, (ii) the *internal dose* is the amount of toxicant absorbed into the body, and (iii) the *biologically effective dose* is the amount of toxicant that reaches the target tissue where the health effect is initiated. Methods for estimating dose can be classified broadly as direct or indirect. The direct measurement of external dose is estimated from measurements of a toxicant in a person's microenvironment with a method such as personal air monitoring; direct measurements of internal dose and biologically effective dose are made with exposure markers. Indirect methods of estimating dose require the use of spatial and temporal models that incorporate toxicant concentration distributions and rely heavily on the responses to questionnaires, information on people's activities, and microenvironmental measurements.

An exposure marker is an environmental toxicant, a metabolite of it, or a product of its reaction with a protein, nucleic acid, or organelle that may be measured in human tissue or body fluids. Exposure markers are important because they effectively integrate all routes and sources of exposure and their measurement provides an estimate of the actual dose of toxicant resulting from exposure. Exposure markers of internal dose may be the unchanged toxicant or its metabolite in human samples, and their measurement provides the most clear-cut evidence of a specific environmental exposure; such markers are most conveniently measured in urine, but blood, breast milk, or other materials may be more suitable. A marker of biologically effective dose is the amount of toxicant or metabolite that has interacted with a target site, and is most commonly measured in peripheral blood cells or in samples of bone marrow, placental tissue, or bronchial lavage. However, an ex-

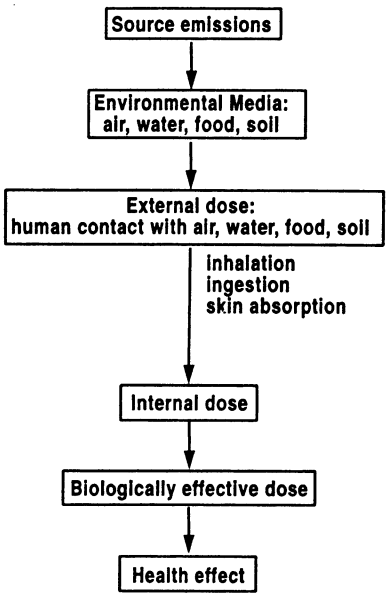

Fig. 1. Exposure and health effects pathway.
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posure marker can be reliably measured and interpreted only when the pharmacokinetics (i.e., rates of absorption, distribution, metabolism, elimination) of a toxicant are understood. Such an exposure marker allows meaningful classification of human exposure to a particular toxicant and increases the power of epidemiologic studies to identify associations between exposure and disease.

Exposure markers are one category of biological markers (biomarkers) that can be measured in the continuum of events between human exposure and disease. The health effects shown in Fig. 1 are broadly classified as early biologic effects and altered structure and function (*3*). The underlying biochemical, molecular, genetic, and immunologic mechanisms of biological markers have been reviewed (*4*).

Recent technical and scientific developments in the area of exposure marker methodology have come from many diverse fields, including instrumentation and separation science, synthetic chemistry in relation to analytical standards, pharmacokinetics, toxicology, and epidemiology. In this article we highlight some major advances in the measurement of exposure markers that have substantially improved the research worker's ability to estimate the internal dose of an environmental toxicant.

Characteristics of Analytical Methods

A valid exposure marker of internal dose is important in efforts to qualitatively document that an exposure has occurred, or to quantitatively measure the magnitude of the exposure. Ideally, an exposure marker to be used as a "dosimeter" should show a defined relation to human exposure over a wide range of toxicant concentrations and should be highly specific for the toxicant in question. Ultimately, the relation between the dose of the toxicant and the effect on human health should be well established.

Applied research into human exposure markers for estimating internal dose is focusing on developing laboratory methods with well-characterized precision, accuracy, detection limits, and analytical specificity. Such a method should be robust and stable over time, with carefully documented scope and limitations, and should have a "reasonable" rate of specimen throughput for examining populations at risk of exposure to a toxicant. Reference limits or intervals must be established for an exposure marker in a population with no known source of exposure. A quality-assurance plan must be in place that should specify calibration and quality-assurance materials to be used in each analytical run. Human specimens must be collected, transported, processed, and stored according to strict protocols designed to minimize contamination from the environment and from collection equipment. Choice of the most appropriate type of human specimen should be based on accessibility, pharmacokinetic information, and the special requirements of the analytical methods.

The concentrations of most toxicants in human specimens are typically 10 to 1000 times lower than in environmental media. Thus, performing reliable measurements on a small amount of human material requires sensitivity that is likely to be at the leading edge of current instrumentation and methods. There are more than 3 million environmental toxicants, many of which are complex chemical structures with related compounds (called congeners). For example, chlorinated dibenzo-*p*-dioxins are a family of 75 different congeners with varying numbers and placement of chlorine substituents around the basic ring structure; the toxicities of the various congeners vary greatly. In guinea pigs, for example, the toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin is 15 000 times greater than that of 2,3,7-trichlorodibenzo-*p*-dioxin. Thus, the identification of the specific congener can be critical for determining the nature of the exposure. At present, the measurement of most organic toxicants requires extensive and scrupulously clean specimen work-up, followed by measurement with gas or liquid chromatography and mass spectrometry. The amount of labor in such methods must be carefully factored into the design of epidemiologic studies because specimen throughput, detection limit, and analytical accuracy and precision contribute to the overall cost.

Exposure Markers vs Indirect Methods of Estimating Exposure

Indirect methods of estimating exposure are used extensively in environmental health studies. These methods rely heavily on mathematical models and population survey information to describe interactions among components of the environment, the toxicant, and the human population. Exposure models are based on time and activity patterns of people living and working in various microenvironments, on distributions of toxicants in each microenvironment, and on the make-up of the sample population. Epidemiologists generally develop exposure indices, derived from exposure models and the best available historic information, to calculate the exposure of each person in the sample population.

With all such indirect methods, exposure-related data on human activity patterns are crucial to the analysis. Time-activity patterns can be obtained by either direct observation, which is labor intensive and expensive, or by questionnaires, the results of which are dependent on human understanding, motivation, and memory. Ideally, if the assumptions and approximations of those using indirect methods are correct, and if those using direct methods have full knowledge of the pharmacokinetics of each toxicant when they measure the biological markers of internal dose, the estimates of exposure derived from the two approaches should show good agreement.

Figure 2 summarizes the results of one such comparison between an exposure index and an exposure marker in assessing the internal dose of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD, henceforth referred to as "dioxin"), a toxic contaminant in the herbicide "Agent Orange" (*5*). Before 1988 the US Air Force (USAF) was using an exposure index to calculate the dose of dioxin that its personnel may have received as a result of spraying Agent Orange during the Vietnam War as part of Operation Ranch Hand in 1962–1971. The USAF exposure index was carefully developed for each of these veterans by using relatively good information on dioxin concentrations in batches of Agent Orange during the spraying periods, the subject's occupation and length of service in Vietnam, and an accounting for the number of gallons of Agent Orange sprayed during the subject's service. This index was the basis for calculating exposure for each of the 1200 Ranch Hand participants enrolled in the study. In 1989 scientists at the CDC measured the serum dioxin concentrations in 620 of the Ranch Hand participants and compared the results with the exposure index values. They found essentially a negative correlation between the measured dose and the calculated dose: Many individuals with a high exposure index had low serum dioxin concentrations, and other individuals with high serum dioxin concentrations had a low exposure index. In this particular instance, the exposure index was abandoned in favor of the serum dioxin concentration as a means of estimating dose.

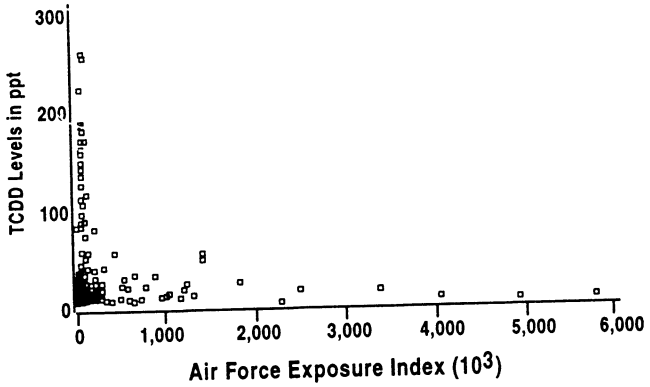

Fig. 2. Serum dioxin concentrations vs the US Air Force exposure index for 620 Operation Ranch Hand personnel.
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In Table 1 we compare questionnaire data used to classify smoking status and data on a marker for measuring human exposure to tobacco smoke. Both sets of data are from studies of smoking cessation during pregnancy conducted by US state health departments in Colorado, Maryland, and Missouri (Kendrick JS, Zahniser SC, Salas N, et al., and Sexton M, Metzger RJ, Stockbauer JA, et al., personal communications). Approximately 5572 women were enrolled in three studies to evaluate the effectiveness of various smoking cessation programs. Several times during the studies, urine specimens were collected from the participants for cotinine measurements. Cotinine is the primary human metabolite of nicotine and can be reliably measured in the urine up to several days after a person's exposure to tobacco smoke. The histogram of cotinine concentrations found among participants in one of the state studies (Fig. 3) showed a good separation between smokers and nonsmokers, with cotinine values above 85 μg/L related to active smoking and values less than 85 μg/L attributable to passive exposure to environmental tobacco smoke. The histograms of cotinine results were similar for the other two states and confirmed a cutoff value of 85 μg/L for the exposure marker. Questionnaire data showed that smokers in groups that received counseling to quit smoking did so at a significantly higher rate than did those in groups that did not receive counseling (Table 1). However, exposure marker data showed no significant difference between the groups; further investigation showed recall biases ranging from 30% to 70% in the three state studies. As a result of this finding, many of the federally funded smok-

Table 1. Comparison of urinary cotinine excretion with questionnaire data in a study of smoking cessation during pregnancy (n = 5572 women in control and intervention groups from three states).

Percentage who quit smoking	Control group	Intervention group	*P*
Questionnaire findings, self-reported	9.5	13.0	0.006
Exposure marker findings, urine cotinine <85 μg/L	5.9	6.1	0.80

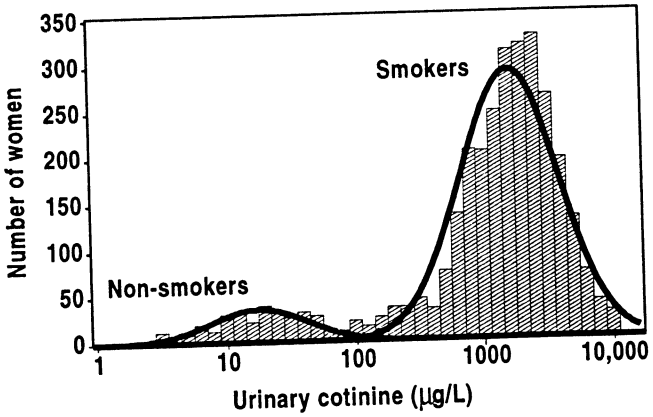

Fig. 3. Distribution of urinary cotinine data from smoking cessation studies.

ing cessation programs are incorporating the measurement of urinary cotinine into their strategies.

The Role of Human Pharmacokinetics in Selecting Appropriate Exposure Markers

An understanding of the pharmacokinetics of a toxicant is crucial to the proper selection and use of an exposure marker for estimating internal dose. Pharmacokinetics is important for judging whether a toxicant, or one of its metabolites, persists long enough after exposure to be measured and for determining the most appropriate biological sample for analysis. The absorption, metabolism, partitioning to body compartments, and elimination of a toxicant can vary with age, sex, diet, hormonal and general health status, and current or past exposures to other toxicants. It is important to note that pharmacokinetics are further complicated if the marker of internal dose being evaluated is the product of the reaction of a toxicant with cellular macromolecules.

Most of the pharmacokinetic data for exposure markers now available are derived from studies with experimental animals that have been given very large doses of a single hazardous substance under controlled laboratory conditions. Apart from intrinsic species differences, the extrapolation of animal data to humans poses serious problems: Animals are likely to have different pharmacokinetic responses to the same toxicant, because doses to which humans are exposed are typically 1000 to several million times lower than doses administered to laboratory animals. In addition, animal studies rarely account for other confounding human exposures such as to alcohol and tobacco smoke.

An example that highlights the importance of human pharmacokinetic data in selecting an appropriate exposure marker involves dioxin. Results of dosing studies of various species of animals during the 1970s and early 1980s indicated that the biological half-life of dioxin is 2–6 weeks (*6*). However, during the mid-1980s, military personnel and veterans were expressing concern that their exposures to dioxin in Vietnam, which may have occurred some 20 years earlier, were responsible for a variety of ill effects (e.g., cancer) in themselves and birth defects in their offspring. The animal data suggested that the direct measurement of dioxin, as an exposure marker of dose, would not be possible because many half-lives would have elapsed. In fact, a histogram of dioxin half-life results, calculated on the basis of serum samples collected 5 years apart from the participants in the Ranch Hand study (*7*), suggests, if one assumes first-order kinetics, that dioxin has an elimination half-life of more than 7 years in humans. Most of the variability in half-life data can be accounted for by the analytical variation in the measurements (CV 20%). More recent (unpublished) calculations of the half-life, based on a much larger sampling of >300 Ranch Hands participants, also yields a half-life estimate >7 years. These half-life calculations allow researchers to reliably measure and interpret serum dioxin concentrations in persons excessively exposed to dioxin more than 30 years ago.

Selection and Storage of Specimens for Testing

Reasons for measuring exposure markers in specimens other than blood and urine include relative availability of tissue (e.g., babies' or children's teeth, autopsy tissue), pharmacokinetic partitioning of toxicant (e.g., adipose tissue for hydrophobic toxicants, bile), and assessment of exposures on a historical basis (e.g., hair). The lack of medically trained personnel needed to perform invasive procedures may also be a consideration (e.g., breath, fingernails). In addition, environmental epidemiologic studies can be designed to examine specific questions that may require special specimens (e.g., breast milk, umbilical cord) related to infant exposures.

According to their chemical and physical properties, toxicants or their metabolites partition to various compartments in the human body. When exposure markers of internal dose are measured in fluid or tissue of one compartment, it cannot be assumed that the results accurately reflect concentrations in the other compartments. In a study of exposure to waste oils sprayed on roads in Missouri, researchers examined the correlation between dioxin concentrations in serum and adipose tissue. Dioxin is a lipophilic compound, and before 1986, adipose tissue was generally accepted as the preferred sample for measuring dioxin in humans. The major disadvantage of using adipose tissue is that it must be removed surgically. The analytical methodology for dioxin in the mid-1980s required 10 g of adipose tissue. At the same time, 450 mL of blood was collected from 50 individuals in the same study. In Fig. 4 we compare the dioxin concentrations measured in adipose tissue with the corresponding values for serum, both sets of samples being adjusted for lipid content (*8*). The excellent correlation spans 2.5 orders of magnitude, from approximately 3 to 1000 parts per trillion, and suggests that serum is a suitable matrix for assessing the dose of dioxin in humans. The practical advantage of this correlation was that it allowed researchers (such as those in the Ranch Hand study) to collect serum rather than adipose tissue samples—an advance that greatly facilitated subsequent studies of dioxin exposure.

For toxicants with short half-lives, the timing of collection is critical to the interpretation of measurement

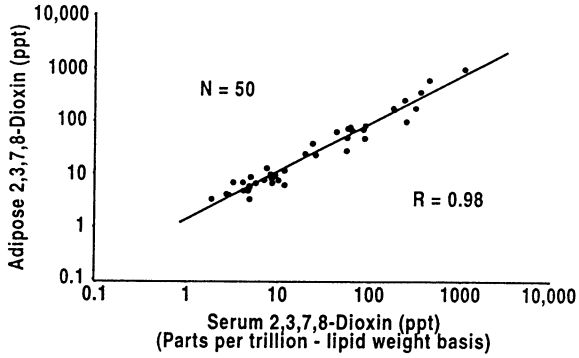

Fig. 4. Correlation between concentrations of dioxin in serum and adipose tissue, from a study in Missouri.

Data expressed in terms of lipid content. Reprinted with the permission of Springer-Verlag New York Publishers (*8*).

data. Preliminary estimates indicate that, in the blood compartment, the half-lives of many of the volatile organic compounds (VOCs) are less than 1 h, indicating that the time of blood collection relative to the time of environmental exposure is crucial to proper sampling. Environmental contamination remains a major problem in collecting specimens to be used in measuring toxic elements. Blood and urine collection containers and collection procedures must be evaluated before use, both for potential contamination and absorption. For example, even though concentrations of lead in blood have been monitored for 20 years or more, contamination remains a major issue in obtaining reliable specimens for blood lead measurements.

The stability of the toxicant in the sample under the storage conditions prior to any measurements must also be examined. In many environmental health studies, specimens are collected prospectively and laboratory measurements are performed years or even a decade after collection. Under storage, toxicants (e.g., VOCs) may diffuse out of the specimens, body fluids may leach toxicants from rubber stoppers or from glass or plastic containers, and frozen human fluids may slowly desiccate out of improperly sealed containers.

Advances in Technology and in Analytical Standards

The measurement of exposure markers for epidemiologic studies has advanced in three areas: (i) analytical detection limits, (ii) technology for specimen throughput, and (iii) synthesis of analytical standards.

With improved detection limits, measurements of exposure markers can be made on smaller specimens; e.g., in 1986, ~250 mL of serum was required to measure dioxin in low parts-per-trillion concentrations (lipid basis) with the high-resolution, state-of-the-art mass spectrometers then available. Since then, technical breakthroughs in electronics or instrument design have resulted in improvements in analytical sensitivity. Currently, dioxin can be measured in 10-mL portions of serum without compromising the detection limit. Thus, researchers can now conduct epidemiologic studies of human dioxin exposures for which previously they would have required special ethical or institutional review, and which would have had low rates of subject participation.

Specimen throughput has been a major problem in measuring exposure markers. Many toxicants and metabolites must be analyzed by isotope-dilution mass spectrometry, which is generally not amenable to rapid specimen throughput. In 1987, a high-throughput method was sought for measuring serum cotinine in the low parts-per-trillion range for use in the third US National Health and Nutrition Examination Survey (NHANES III) as a possible exposure marker for obtaining national estimates of passive exposure to tobacco smoke. NHANES III, a large cross-sectional survey of American households, started in 1988 and will continue through 1994. For this survey, data are collected from an extensive medical history, physical examination, and blood and urine specimens from each participant (*9*). It is expected that cotinine measurements will be made on a total of 23 000 survey participants. Immunoassay technology has the specimen throughput required to perform the measurements and is acceptable for distinguishing between smokers and nonsmokers, but it lacks the analytical sensitivity to reliably measure cotinine concentrations <5 μg/L, a cut-off necessary to measure passive exposure to environmental tobacco smoke. High-resolution gas chromatography-mass spectrometry provides a lower detection limit for cotinine, approximately 5 ng/L in 1 mL, but the anticipated throughput of fewer than 10–15 specimens per day is unacceptable for the scale of this study. Fortunately, a technology that combines high-performance liquid chromatography (HPLC) with atmospheric pressure chemical ionization tandem mass spectrometry (APCI MS-MS) has been developed. The use of a short, high-efficiency (3 μm) HPLC column with an autosampler allows for completion of the entire HPLC-mass spectral analysis in 2 min. A tandem mass spectrometer with dual monitoring of specific parent-daughter ion pairs for quantitation provides the necessary high degree of specificity for the assay. Currently, this technology is being used at CDC to measure cotinine in serum in approximately 100 specimens per day with a detection limit of 30 ng/L. The specimen-extraction and cleanup steps have now become rate-limiting.

Recent advances in the synthesis of many toxicants and metabolites, and of isotopically labeled analogs, have greatly improved the quantitative analysis of exposure markers. A major problem in the analysis of cotinine in biological fluids has been the selection of an appropriate internal standard. A variety of such standards has been used, including 2-phenylimidazole, methylprylone, lidocaine, diphenylamine, ketamine, 5-methylcotinine, *N*-ethylnorcotinine, methylanabasine, amphetamine sulfate, and norephedrine. The choice of internal standard was sometimes inappropriate; e.g., with lidocaine, the resulting measurements show substantial bias and imprecision (*10*). Because lidocaine is both substantially more lipophilic and more basic than cotinine, it must be added to the sample extract because it does not compensate for variations in extraction efficiency. In general, only an analog that is structurally close to a toxicant, and that has similar chemical and physical properties, is appropriate as an internal standard. When mass spectrometric techniques are used, an isotopically labeled analog is the best choice for the internal standard. For mass spectrometric analyses of cotinine, the trideuterated analog, cotinine-methyl-D3, was synthesized and is now generally used as the internal standard. It is commercially available and has been incorporated into the NHANES cotinine studies. However, a limitation of this approach is the need for isotopic purity of the labeled analog; i.e., none or very little of the parent substance should be present.

Exposure Markers in Environmental Health Studies

Preliminary findings on cotinine concentrations in the first 800 participants tested in the NHANES III study showed that everyone had detectable serum concentrations of cotinine (*11*). A bimodal frequency distri-

bution of cotinine values was observed, extending over 4 orders of magnitude from 0.03 to 650 μg/L. NHANES III was designed as a two-phase study with "national probability samples": one phase from 1988 through 1991 and the second from 1992 through 1994. A comparison of serum cotinine concentrations between the first and second national samples within NHANES III will provide a rare opportunity to assess the effectiveness of public health efforts to reduce exposure to tobacco smoke in the US. In addition, representative sampling of various racial, ethnic, socioeconomic, and age groups will provide important data on exposures in these populations.

Measuring exposure markers in the general population on a national and international basis is one of the most effective ways for environmental health programs to identify new toxicants and their sources, determine the prevalence and levels of exposure, and monitor trends of exposure to toxicants. Measurements of blood lead in 10 000 participants of NHANES II, conducted between 1976 and 1980, showed average concentrations of approximately 16 μg/dL in the early phases of the study (*12*). In the mid-1970s, catalytic converters were incorporated in the exhaust systems of several models of American automobiles to meet air emission standards for CO and NO_x/SO_x. Because lead from gasoline tended to destroy the catalyst in the emission control device, automobiles requiring unleaded gasoline began to increase their market share. As shown in Fig. 5, average blood lead concentrations in the US population decreased steadily from mid-1978, paralleling the reduction in the amount of lead added to the gasoline (*12*). These data pointed to lead in gasoline as a major source of lead exposure in the US and stimulated federal legislation to remove the remaining lead from gasoline. Preliminary data from NHANES III (1988–1994) indicate that participants have substantially lower lead concentrations than seen at the end of NHANES II. NHANES III data for blood lead will be used to estimate the prevalence of lead poisoning among young children exposed to leaded paint and to select targets for primary lead poisoning prevention activities. NHANES III data will also be collected on urinary cadmium excretion and serum selenium concentration. The results will provide the first national estimates of exposures to these two elements.

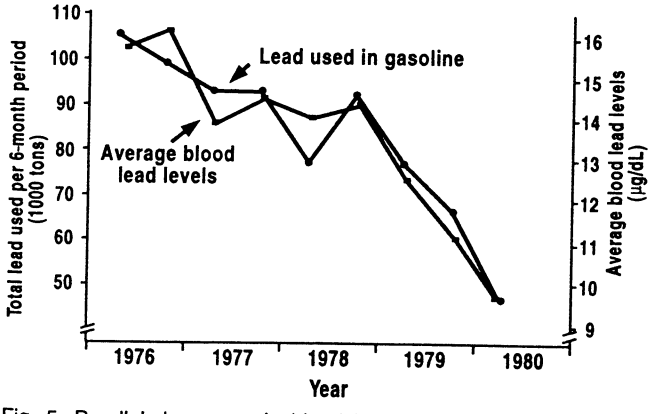

Fig. 5. Parallel decreases in blood lead values observed among NHANES II participants and the amount of lead added to gasoline (1976–1980).

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A subset of 1000 participants in NHANES III is being monitored for urinary concentrations of 12 pesticides or their metabolites and blood concentrations of 32 VOCs. The results will provide reference population data for these 44 toxicants as they relate to age, gender, race, urban vs rural status, and region of the country. Tables 2 and 3 list the VOCs and the pesticides and their metabolites being measured in the survey. Preliminary results show that 16 VOCs and 6 pesticides or their metabolites can be identified in 50% or more of the reference population (Needham, Hill, Ashley, personal communication). These background or reference range data have already been applied to various public health emergencies involving exposures to toxicants. When complete, these findings will provide a valuable database for assessing exposures to these two categories of toxicants among individuals in various occupational settings, for assessing exposures of populations residing around hazardous waste sites, and for establishing regulations that limit the use of certain pesticides.

Interpretation of Exposure Marker Measurements

An important objective in environmental health is to establish the relation between exposure to a hazardous

Table 2. NHANES III: 32 VOCs quantified in priority toxicant reference range study.

Benzene	1,1-Dichloroethane	Methylene Chloride
Toluene	1,2-Dichloroethane	Chloroform
Styrene	1,1-Dichloroethene	Carbon Tetrachloride
Ethylbenzene	*cis*-1,2-Dichloroethene	1,2-Dichloropropane
1,2-Xylene	*trans*-1,2,-Dichloroethene	Bromoform
1,3-Xylene	1,1,1-Trichloroethane	Dibromomethane
1,4-Xylene	1,1,2-Trichloroethane	Bromodichloromethane
Chlorobenzene	Trichloroethene	Dibromochloromethane
1,2-Dichlorobenzene	1,1,2,2-Tetrachloroethane	Acetone
1,3-Dichlorobenzene	Tetrachloroethene	2-Butanone
1,4-Dichlorobenzene	Hexachloroethane	

Table 3. NHANES III: List of selected chemicals measured in human urine and their pesticide precursors.

Chemical measured	Pesticide precursor
1-Naphthol	Naphthalene, carbaryl
2-Naphthol	Naphthalene
Isopropoxyphenol	Propoxur
Carbofuran phenol	Carbofuran
3,5,6-Trichloro-2-pyridinol	Chlorpyrifos
2,4-Dichlorophenoxyacetic acid (2,4-D)	2,4-D
Pentachlorophenol	PCP, HCB, γ-BHC
2,4,5-Trichlorophenol (2,4,5-TCP)	2,4,5-TCP; 1,2,4-trichlorobenzene; γ-BHC; HCB
2,4,6-Trichlorophenol (2,4,6-TCP)	2,4,6-TCP; 1,3,5-trichlorobenzene; γ-BHC
2,5-Dichlorophenol (2,5,-DCP)	p-Dichlorobenzene
2,4-Dichlorophenol (2,4-DCP)	m-Dichlorobenzene
4-Nitrophenol	Methyl and ethyl parathion; nitrobenzene

substance and the resulting morbidity or mortality. When acute health effects or death result from high-dose exposures to toxicants, exposure measurements of internal dose can establish dose–disease thresholds for specific toxicants. Public health emergencies associated with chemical explosions or other unintentional releases of hazardous substances have provided some valuable information on toxic thresholds (*13, 14*). However, in the absence of an acute episode of high-dose exposure, subsequent disease or death is difficult to relate to a low-level, chronic exposure to a specific toxicant. To develop dose-disease relationships, researchers must first carefully search out populations that have experienced a wide range of exposure to specific toxicants. Next, to establish toxicant doses, they must use reliable methods to measure exposure markers in biological samples from the various populations. Finally, they must develop a plan that will allow them to look for health outcomes over time, taking into account potentially confounding factors such as age, sex, lifestyle, and exposure to other toxicants.

In Fig. 6 we summarize the median serum dioxin concentrations in selected populations examined since 1985; values are plotted on a logarithmic scale. The findings reflect various types of long-term or acute situations: exposure of residents to hazardous waste in the roads of Missouri (*15*); exposure of military personnel to Agent Orange (*16, 17*); exposure of civilians to herbicide sprayers (*18*); exposure of workers in chemical plants (*19*); and exposure from an industrial explosion that caused a cloud of dioxin to cover part of the town of Seveso, Italy (*20*). The serum dioxin concentrations ranged from approximately 5 parts per trillion for individuals with no known source of exposure to 50 000 parts-per-trillion (lipid basis) for some people in Seveso. Three of these studies—of chemical plant workers, Ranch Hand veterans, and Seveso residents—have a longitudinal component. Hence, at 5-year intervals, participants in the Ranch Hand study are given a detailed physical examination, and their serum dioxin concentrations are remeasured.

Collectively, the exposure marker measurements performed for such studies serve to provide the basis of a common quantitative estimate of dose. In addition, the data in Fig. 6 have been useful for interpreting serum dioxin measurements in other public health risk assessments. For example, there has been concern for the safety of people who moved into contaminated areas around Seveso after the 1976 explosion and lived there for several years. However, laboratory measurements of serum dioxin in these immigrants showed that their dioxin concentrations were compatible with background values observed in people with no known source of exposure. A current example involves measurement of serum dioxin in residents of Jacksonville, AR, who live around the Vertac Plant that previously made di- and trichlorophenoxyacetic acid (active ingredients in Agent Orange). Starting in 1994, stockpiles of waste from the abandoned site are scheduled for burning in a large incinerator constructed on the site. Jacksonville residents living near the site will have their serum dioxin concentrations measured before and after the waste incineration, to establish their current dioxin burden and to monitor any changes occurring after the incineration.

To broaden the human dioxin database even further, the International Agency for Research on Cancer (IARC) is beginning to collect serum for dioxin measurement from the 20 different cohorts in 10 countries in its worldwide dioxin registry. The information generated in these studies will provide an important and much improved database for all future risk assessments, epidemiologic studies, and public policy activities involving dioxin.

A more general observation regarding the future use of exposure markers in environmental health can be inferred from the ongoing work with dioxin. Measurements made on several thousand people, including many with no known direct source of exposure, show that virtually all have detectable concentrations of dioxin in their serum. The large public expense of entering individuals into long-term exposure registries to monitor health effects or mortality requires that only persons or populations with excessive exposures should be followed over time. Since exposure markers offer a very effective technique to assess individual exposures for long-term follow-up activities, it is evident that such markers will be used widely in the future.

Quality Assurance Among Laboratories

It is important to ensure that measurements of exposure markers performed in multiple laboratories with

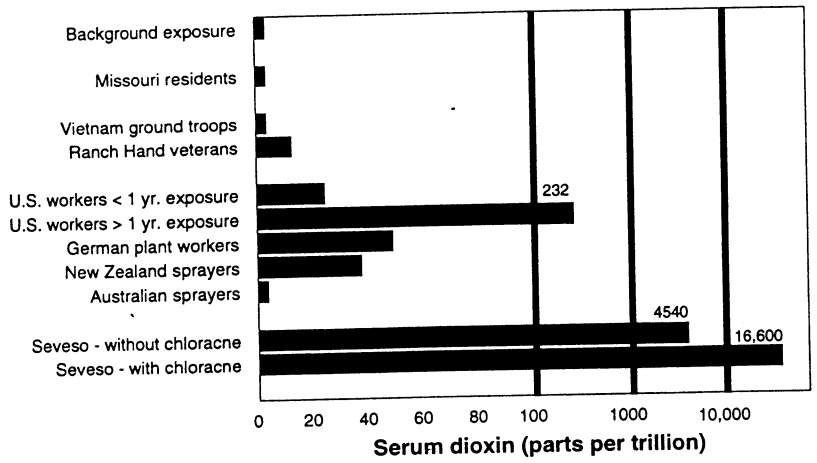

Fig. 6. Median serum dioxin concentrations in selected populations (lipid basis).

different instruments, controls, and operators are comparable over space and time. As specific guidelines are established for safe exposures to hazardous substances, strict requirements must be introduced for assuring the quality of exposure marker measurements.

For more than a decade, several hundred laboratories in the US have been screening individuals for subclinical lead poisoning. Initially, children were screened for increased erythrocyte protoporphyrin (or zinc protoporphyrin); those with high values received a blood lead measurement to determine whether exposure to lead was responsible for the increase. For children, the pre-1985 blood lead concentration at which intervention was suggested was ≥25 μg/dL. However, in 1991 new guidelines were established for a national program to prevent lead poisoning in children, and the intervention cutoff value for blood lead was revised downward to 10 μg/dL (*21*). In addition, blood protoporphyrin was shown to be insufficiently sensitive as a marker for identifying children with blood lead concentrations <25 μg/dL, so that measurement of blood lead is now recommended as the primary screening method. Because the detection limit of some of the older technology for measuring blood lead concentrations was not low enough for screening, instrument manufacturers are developing a new generation of analyzers with lower detection limits, improved specimen throughput, and lower costs per test.

In Fig. 7 we show data from a quarterly proficiency-testing program (*22*) designed to determine the distribution of blood lead results from 173 laboratories for a pool with a mean target value of 6 μg/dL. On the basis of a single blood lead measurement, five of the laboratories have misclassified this sample as having a value of ≥10 μg/dL. Three laboratories reported no detectable value. The overall distribution of laboratory results suggests a considerable need for improvement. In response to this need for improved laboratory performance, a national Blood Lead Laboratory Reference System (BLLRS) has been initiated, which includes new blood lead reference materials developed at CDC.

Efforts are being made to expand the standardization process to other exposure markers. Guidelines have been established for acceptable concentrations of cadmium in factory workers. Proficiency-testing programs are being used in developing quality-assurance materials for blood cadmium measurements. Other efforts have focused on assuring the comparability of measurements of other toxicants in human samples. For example, the World Health Organization (WHO) sponsored several international laboratory surveys to assess the comparability of measurements of dioxin and other polychlorinated toxicants in laboratories that perform isotope dilution-mass spectrometry measurements of human blood and breast milk samples (*23*). A not-unexpected finding from these surveys is that lipid measurements, used as a basis for comparison of toxicant concentrations, are one of the largest sources of variation among participating laboratories.

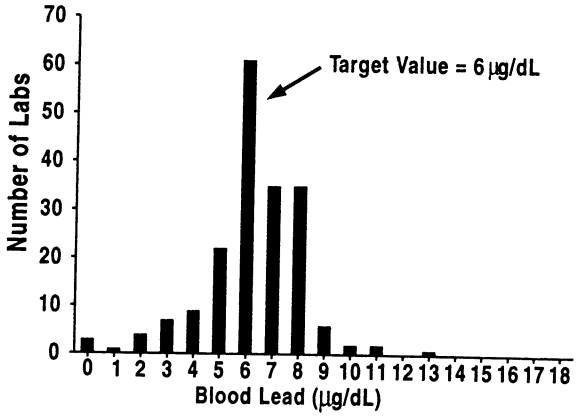

Fig. 7. Distribution of blood lead values from 173 laboratories participating in a proficiency testing program.

Recommendations for the Future

The most critical need in each of the areas we have discussed is for more data on human samples so that measurements of exposure to environmental toxicants can be interpreted reliably. Because of the relative lack of biological exposure markers for most toxicants and because of the high costs of direct measurements, the use of indirect methods for assessing exposure to environmental toxicants will continue. However, when investigators use indirect methods to estimate the dose of a toxicant for which there is a reliable human exposure marker, they should validate the exposure index, questionnaire, and other models against the exposure marker in an effort to ensure that the assumptions or conclusions regarding exposure are correct.

The use of laboratory animals in toxicity studies and in studies of the pharmacokinetics of toxicants will continue. However, there are major differences in pharmacokinetics between species of animals, and, more important, between humans and other species of animals. As part of the validation of any exposure marker for humans, researchers must continue to gather pharmacokinetic information on human subjects. Most of this work will involve populations with high rates of occupational exposures or those affected by acute, unplanned releases of toxicants. Some relevant information may also be derived from cases of poisoning, either intentional (self-inflicted) or unintentional (accidental).

We anticipate that researchers will continue to use many different types of human tissue to measure exposure markers because much of the current applied research in environmental health involves epidemiologic studies designed to test very specific hypotheses related to exposure. However, as exposure markers gain wider use for monitoring populations, the accessibility of the samples used will become an important practical consideration. In planning an epidemiologic study based on sampling an unusual tissue or fluid, researchers should also consider collecting blood or urine so that possible associations between samples and measurements can be elucidated.

Although rapid advances in the hardware and software for laboratory measurements of exposure markers are being made, improvements are needed in sample preparation. Typically, for nonvolatile organic compounds, an

initial extraction with an organic solvent is commonly followed by concentration by solvent evaporation, before the sample is subjected to chromatography and instrumental analysis. Safe handling and safe disposal of solvents, and the labor-intensity of many methods, lower sample throughput and increase the overall cost of the measurements. Attention must therefore be given to developing better automated sample preparation techniques and improved extraction technology. Supercritical fluid extraction and chromatography is an example of a new technology meriting attention in this context.

A greater emphasis must be placed on establishing reliable exposure marker data, both on reference populations and on populations with presumed high exposures, so that measurements can be better interpreted. Within the US, the National Human Exposure Assessment Survey (NHEXAS) has been proposed as a vehicle to examine routes, magnitudes, and frequencies of human exposures to important environmental toxicants (*24*). An additional role of NHEXAS is to explore options for measuring exposure markers in relevant populations. During investigations of populations that may have been excessively exposed to toxicants, epidemiologists should be encouraged to collect human specimens suitable for future measurements. Banked specimens serve to capture a specific moment in the exposure life of a person or population and may be analyzed years later, thus providing valuable links between exposure and health effects (*25*).

Major advances in assuring the quality of exposure marker measurements among a network of laboratories will probably coincide with the establishment of more useful exposure limits for other toxicants. An additional catalyst in the US will be the implementation of the Clinical Laboratory Improvement Act of 1988 (CLIA). Laboratories performing measurements and reporting results for exposure markers will have to participate in an appropriate proficiency-testing program and meet corresponding criteria for analytical performance.

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